Extended summaries Neurotox '98

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Can selective peptides be combined efficiently with agrochemicals? A new approach to insect control

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Abstract: Sodium channels have been a major target for the development of insecticides such as synthetic pyrethroids. However, insecticides currently available induce resistance and present limited selectivity to insect pests. Molecular and biochemical studies, as well as binding experiments using radiolabelled neurotoxins, have shown that sodium channels expressed in various insect orders must be structurally and pharmacologically different. At least three groups of peptide neurotoxins derived from scorpion venom are highly active on insects and very weakly or practically inactive on mammals. It is proposed that various insecticides are examined for possible cooperative interactions with the peptide toxins highly active on insects, and pairs of ligands are identified that will increase the selectivity not only between mammals and insects but also between different pest and non-pest insects. This is feasible on the basis of the differential allosteric modulations observed between LqhαIT, an α-toxin highly active on insects, and brevetoxin on locust *versus* cockroach and rat brain sodium channels. Moreover, combination of LqhαIT with the pyrethroid deltamethrin increased the binding of [^{125}I] LqhαIT by more than 1.8-fold, and the combined presence of brevetoxin further increased the binding. Such allosteric modulation may provide a new approach to increase the selective activity of pesticides on target organisms by simultaneous application of allosterically interacting drugs, designed on the basis of the selective peptide toxins.

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INTRODUCTION

The voltage-gated sodium channels are critical elements in nerve excitability and serve as the initial target for many toxins developed by a wide variety of venomous animals, including scorpions, spiders and sea anemones. Sodium channels have been a major target for the development of insecticides, such as synthetic pyrethroids.[1] However, insecticides currently available present limited selectivity to insect pests, induce resistance in the pest insects and may disturb the delicate balance between natural predators and pests. Thus, a search for new targets and highly selective insecticides is an important goal in insect control management. Information on the variability in the structure of insect sodium channels and ligands which are highly active on insects may offer a new approach for the strategy of insect control.

Diversity of sodium channels in various insects

We have previously shown, using selective immunoprecipitation of insect sodium channels by anti-peptide antibodies, that sodium channel alpha subunit proteins from various insect neuronal membranes are similar in their biochemical properties to rat brain sodium channels. Sodium channels of locust, cockroach, blowfly head and lepidopteran larvae revealed apparent molecular masses of 245–260 kDa, which were devoid of a disulfide-linked smaller subunit, in contrast to rat brain sodium channels.[2,3] Cloning of *Drosophila* sodium channel,[4] partial proteolysis of various insect sodium channel proteins[3] as well as binding experiments using radiolabelled neurotoxins[5–7] have shown that sodium channels expressed in various insect orders must be structurally and pharmacologically different. Binding studies using [^{125}I] LqhαIT (Lqh – *Leiurus quinquestruatus hebraeus;* IT – insect toxin), the scorpion α-toxin most active on insects, and competition binding studies with several scorpion toxins and the sea anemone toxin ATX II demonstrated that the affinities of the different toxins to locust and cockroach neuronal membranes are different, suggesting structural differences in the sodium channel receptor sites.[6] Dissociation rate constants of bound [^{125}I] AahIT (Aah (called also AaH) – *Androctonus australis* Hector), the excitatory insect selective toxin, in several different insect neuronal membranes revealed k_{off} values in the range

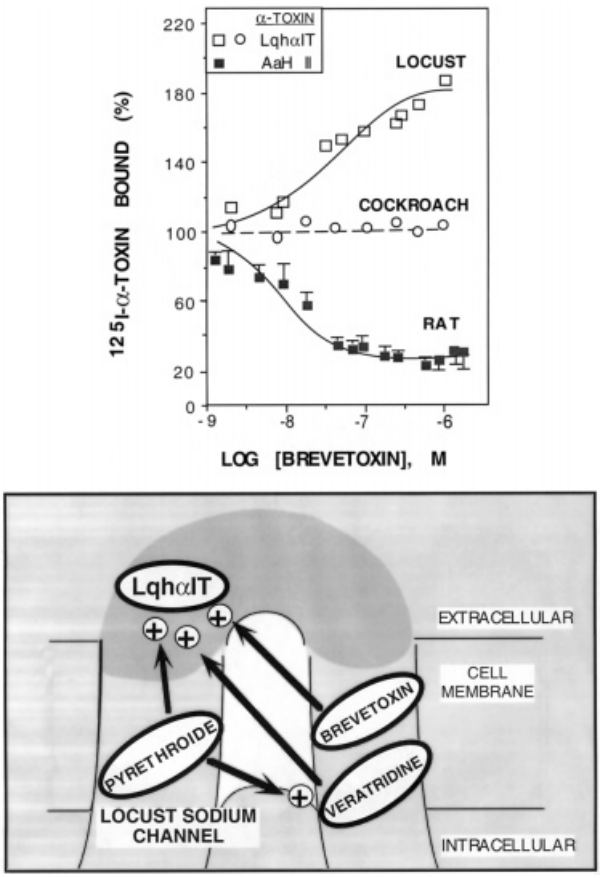

Figure 1. Allosteric interactions among several neurotoxin receptor sites on sodium channels. Interactions between the lipid soluble polyether toxin, brevetoxin PbTx-1, and scorpion α-toxins on rat brain, cockroach (*Periplaneta americana*) and locust (*Locusta migratoria*) sodium channels (Top). The lower panel presents a cartoon of a section through sodium channel α-subunit to demonstrate several allosteric interactions observed by binding studies on the locust sodium channel. The ovals represent the distinct neurotoxin receptor sites as indicated. The arrows indicate the allosteric modulation by binding of one ligand to its receptor site on the binding of the another ligand, linked by the arrows. (+) sign indicates a positive co-operative effect. From References 6 and 21.

of 1.33×10^{-3} (in cockroach) to $98.8 \times 10^{-3} s^{-1}$ (in *Spodoptera* larvae), further indicating that the different insect sodium channels must be structurally distinct.[7] These differences provide the possibility of targetting different sodium channels by selective ligands.

Diversity in sodium channel ligands

At least three groups of peptide neurotoxins derived from scorpion venom are highly active on insects and very weakly or practically inactive on mammals.[8–10,23] The scorpion α-toxin LqhαIT is highly active on insects,[6,10] and the two anti-insect selective toxins, the excitatory (AahIT) and depressant (LqhIT2),[8,11] bind to distinct, high-affinity receptor sites on insect sodium channels and modify their activity differently.[5,6,10,12–16] The high activity and selectivity of the peptide toxins to insects provide the possibility to use them as leads to develop highly selective insecticides. However, a detailed functional analysis of these toxins and an understanding of the structural basis of their selectivity to insects must be obtained as a prerequisite for modifying the peptides in a rational manner. These studies have started with the recent functional expression and analysis by mutagenesis of LqhαIT[16–19] and the depressant and excitatory toxins by Gurevitz and co-workers (Ref 22 and unpublished).

Combinations of ligands

The proposed approach takes advantage of the availability of many ligands that specifically target voltage-gated sodium channels in general, and insect sodium channels in particular. At least seven distinct classes of neurotoxins have been designated, on the basis of physiological activity and competitive binding studies, that bind to several distinct receptor sites on the sodium channel protein.[20] Although the sodium channel receptor sites are topologically separated, there are strong allosteric interactions among them. We have shown that the lipid-soluble sodium channel activators, veratridine and brevetoxin, reveal divergent allosteric modulation of the binding of scorpion α-toxins at homologous receptor sites on mammalian and insect sodium channels.[21,23]

Brevetoxin reveals negative allosteric interaction with AaH II on rat brain sodium channels, but increases the affinity of LqhαIT to locust sodium channels, and has no significant effect on LqhαIT binding in cockroach neuronal membranes (Fig 1). The differences suggest functionally important distinctions between these channel subtypes.

In this approach it is proposed to examine various insecticides, including pyrethroids, for possible co-operative interactions with the peptide toxins highly active on insects, and to identify pairs (or more) of ligands that will increase the selectivity not only between mammals and insects but also between different pest and non-pest insects. This is feasible on the basis of the differential allosteric modulations observed above. To exemplify this approach, the scorpion α-toxin LqhαIT was combined with deltamethrin. As expected, the binding of [^{125}I]LqhαIT was increased by more than 1.8-fold by the pyrethroid, and the combined presence of veratridine and/or brevetoxin further increased the binding (Gilles and Gordon, unpublished). The allosteric modulation may provide a new approach to increase selective activity of pesticides on target organisms by simultaneous application of allosterically interacting drugs, designed on the basis of the selective toxins.

The most recent advances in cloning, characterization and functional expression of new scorpion toxins highly active on different pest insects, the structural and mutagenesis studies that are currently in progress may provide a new momentum to the clarification of the structural basis of insect selectivity of the peptide toxins and enable rational drug design of highly selective insecticides.[22] The combined approach, using pairs of the novel ligands and known agrochemicals, may enable significant reduction in the amount of insecticides used in practice and may aid in

preventing the development of resistance in pest insects.

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Characterisation of the relationship between binding sites for imidacloprid and other nicotinic ligands in insects

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Abstract: Radioligand binding studies using the neurotoxins α-bungarotoxin, epibatidine, imidacloprid (IMI) and methyllycaconitine reveal heterogeneity at the level of the nicotinic acetylcholine receptor (nAChR) in membranes from the peach potato aphid *Myzus persicae* (Sulzer) and further suggest the presence of more than one ligand binding site per nAChR. These sites are able to interact allosterically with each other. Of particular interest, [^{3}H]IMI has over an order of magnitude higher affinity in membranes of hemipteran pest species than in non-hemipteran insects, which may help explain why IMI is particularly effective for the control of sucking pests.

Keywords: *Myzus persicae*; nicotinic acetylcholine receptor; imidacloprid; methyllycaconitine; epibatidine; radioligand binding

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